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TITLE: Impact of C-neu/erbB2 on Estrogen and Estrogen Receptor Alpha-Dependent Proliferation of Mammary Ductal Epithelial Cells

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**13. ABSTRACT (Maximum 200 Words)**

The objective of our research is to examine the expression patterns of estrogen receptor (ERα), progesterone receptor (PR) and C-Neu in mammary glands of wild type and C-Neu transgenic mice during various developmental states and identify the relationships between these expression patterns to cells undergoing proliferation. In previous studies, we demonstrated that there were differences between the mammary glands of wild type and C-Neu mice with regard to their expression patterns of PR. And were apparent as early as six weeks of age. Our present studies reveal that mammary glands of c-neu mice contain abnormal structures with a high rate of proliferation and also that this is ovarian steroid/estrogen independent and detectable as early as 6 weeks of age. The average onset of mammary tumors in c-neu mice is approximately 30-32 weeks Yet, our studies, so far, indicate that c-neu dependent alterations in ovarian steroid hormonal regulation of mammary epithelial cells represent early events and not a late phenomenon associated with tumor progression We propose that estrogen independent proliferation may be intrinsic to mammary cells that over express c-neu. If so, both blocking erbB2 activity and the use of appropriate SERMS may be more beneficial in the clinical management of c-neu cancers.

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## Introduction

Signaling by the sex steroids, estrogen and progesterone, through their cognate receptors, is essential for mammary gland morphogenesis. As such, ductal growth during puberty requires estrogen receptor alpha (ERα) and not progesterone receptor (PR) while lobular-alveolar growth during pregnancy requires PR. The growth promoting effects of these steroids are believed to be mediated by growth factors that signal through the family of erbB receptors, such as C-neu/erbB2. We have found that in transgenic mice over-expressing C-Neu (1), ductal growth during puberty is compromised without any gross impairment in lobulo-alveolar growth during pregnancy (2). Normal mammary glands consists of various epithelial subtypes and the distribution of ERα, PR and C-Neu are heterogeneous in the epithelium and appropriate signaling through hormones and growth factors require cell-cell interactions. Accordingly, we believe that (a) the individual and combined effects of ERα, PR and/or C-Neu (in conjunction with other erbB receptors) depends on the mammary epithelial sub-type and the interactions among these receptors and (b) the net outcome of these interactions is to direct the developmental fate of the various epithelial sub-classes towards ductal or lobular morphogenesis. To test this we are examining the expression patterns of ERα, PR and C-Neu in mammary glands of wild type and C-Neu transgenic mice during various developmental states and identifying the relationships between these expression patterns to cells undergoing proliferation.

## Body

The tasks outlined in the approved statement of work are as follows:

(1) To examine the expression patterns of ER, PR and C-neu in mammary glands of wild type and C-neu transgenic mice during various developmental states and identify their relationships to cells undergoing proliferation; (2) To examine the growth patterns of mammary glands of C-neu transgenic mice upon serial transplantation.

All the research accomplishments reported cover the period of sept.2002 to august 2003.

Analyses for cell proliferation in mammary glands of wild type and C-neu transgenic mice during various developmental states. In these experiments, we used BrdU labeling as an index for analyses of proliferation. In the mammary glands of pubertal mice, intense mitotic activity resides in unique structures called terminal endbuds (TEB's) (3) and consistent with this, TEB's of wild type mice contain a high percentage of cells labeled with BrdU (Fig.1, panel b) while the mature ducts, known to be relatively quiescent, have few labeled cells (Fig.1, panel a). The TEB's in mammary glands of pubertal c-neu mice also exhibit high rate of cell proliferation with the distinction that their structures are abnormal (Fig.1, panel's e and f). Similar to the wild type mice, the mature ducts in C-en mice also have low proliferation (Fig.1, panel c) while abnormal ducts had a high rate of proliferation. (Fig.1, panel d). The number of BrdU positive cells in the various structures in the two strains of mice is presented in Fig.2.

As reported previously (2), in contrast to adult wild type mice, in mammary glands of young adult c-neu mice ductal growth is compromised (12 weeks old) such that they still contained TEB's. As found with pubertal mice, these TEB's were also abnormal and exhibited a high rate of cell proliferation (Figs.2 and 3).

To examine cell proliferation during pregnancy we analyzed tissues from early pregnant (day 6 of pregnancy) and late pregnant (day18 of pregnancy) mice. As well known, with

the onset of pregnancy, there was an increase in cell proliferation in mammary glands of both wild type and c-neu mice which declined during late pregnancy (Fig.2). However, in c-neu mice, during early pregnancy, the proliferation was greater than that observed with wild type mice. (Fig.4). On the other hand, similar to wild type mice, there was a reduction in cell proliferation during late pregnancy. (Fig.4).

Cell proliferation in TEB's of c-neu mice is ovarian independent. It is well known that estradiol signaling through ERα is essential for cell proliferation associated with TEB's and also their maintenance (4,5)). Having found that ductal growth was compromised in mammary glands of c-neu mice, as part of specific aim 1, we had proposed to examine the mammary glands of c-neu transgenic mice for their ability to respond to estradiol with cell proliferation .To this end, we depleted the circulating levels of endogenous estrogen with ovariactomy and examined its impact on cell proliferation. In mammary glands of wild type pubertal mice, ovariactomy causes the disappearance of the majority of TEB's (6)) due to cessation of proliferation such that only mature ducts are present in these mammary glands; these, as expected, have low BrdU labeling index (Fig.2). In contrast, ovariactomy did not have a significant effect on cell proliferation in the TEB's of c-neu mice (Fig.5, panel b and Fig. 2).

As found with pubertal mice, proliferation in the endbuds of adult mice also appeared to be estrogen independent such that it was unaffected by treatment with antiestrogen, ICI-182, 780. (Fig.5. Panel d)

**Key Research Accomplishments**

1.Mammary glands of c-neu mice contain abnormal structures with a high rate of proliferation.

2.Cell proliferation in mammary glands is ovarian steroid/estrogen independent and is detectable as early as 6 weeks of age.

**Reportable Outcomes:**

Funding applied for based on work supported by this award.

**Conclusions**

The average onset of mammary tumors in c-neu mice is approximately 30-32 weeks (1). Yet, as shown in this report, in mammary glands of C-neu mice, ovarian independent proliferation appears to be an early event detectable as early as 6 weeks of age indicating that it is not a late phenomenon associated with tumor progression These observations have direct relevance to human mammary tumors that over express erbB2 /HER-2 which do not respond to endocrine therapy .It is generally presumed that the inability of these tumors to respond to endocrine therapy is because they are in late stages of progression. Our studies suggest that estrogen independent proliferation may be intrinsic to mammary cells that over express c-neu. . This leads us to propose that a higher degree of erbB2 expression seen in early phases of breast cancer may not be sufficient for progression from a benign to a malignant phenotype.

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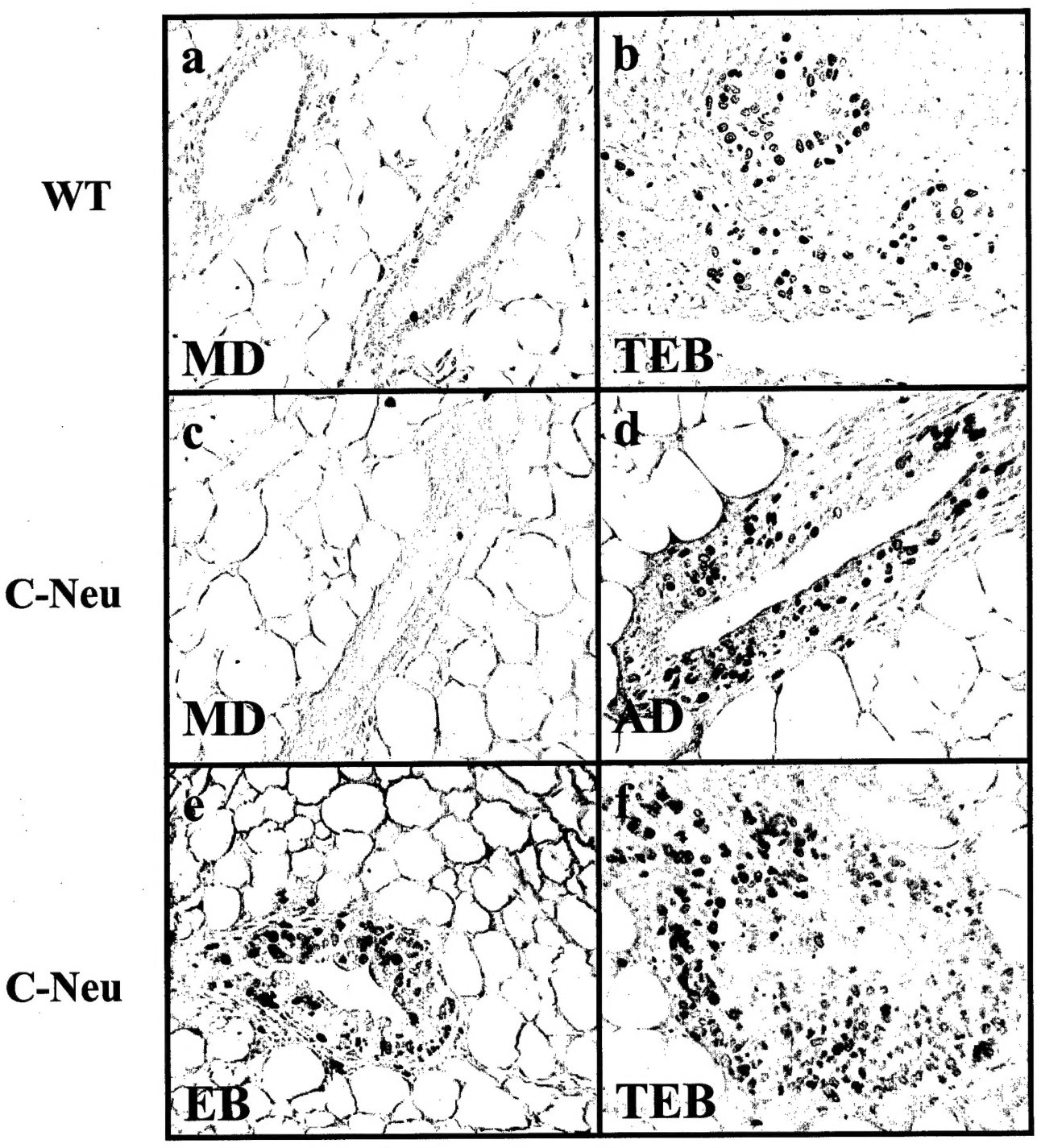


Fig. 1 Analyses for cell proliferation in mammary glands of pubertal wild type and c-neu transgenic mice. Mammary glands from wild type (a, b) and c-neu transgenic mice (c-f) were analyzed for immunoreactive BrdU as described previously (4). Magnification 400 X. MD: Mature ducts; TEB: Terminal end buds; AD: Abnormal duct; EB: End bud.

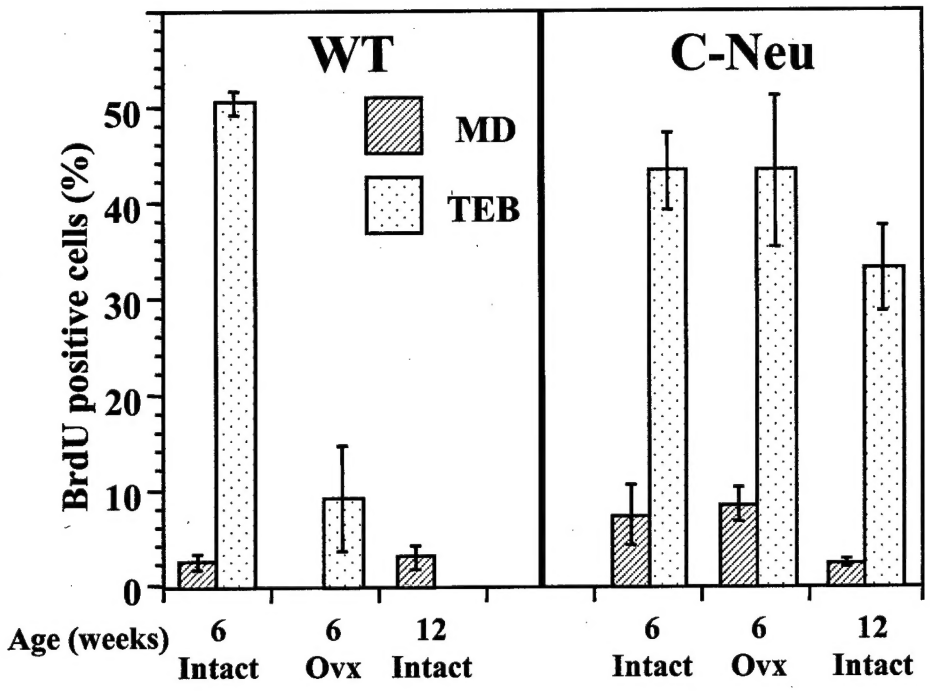


Fig. 2 BrdU positive cells in mammary glands from intact and ovariactomized pubertal (6 weeks) wild type and c-neu mice and intact adult (12 weeks old) wild type and c-neu mice. BrdU positive cells were analyzed as described previously (4). The data is presented as percentages (mean ±S.E.M.) in the different morphological structures: TEB, terminal end bud; MD, mature ducts. For each experimental group, three mice were analyzed and mammary glands for each mouse were analyzed in triplicate. The percentage of immuno-positive cells was obtained by counting a minimum of 500 cells per gland.

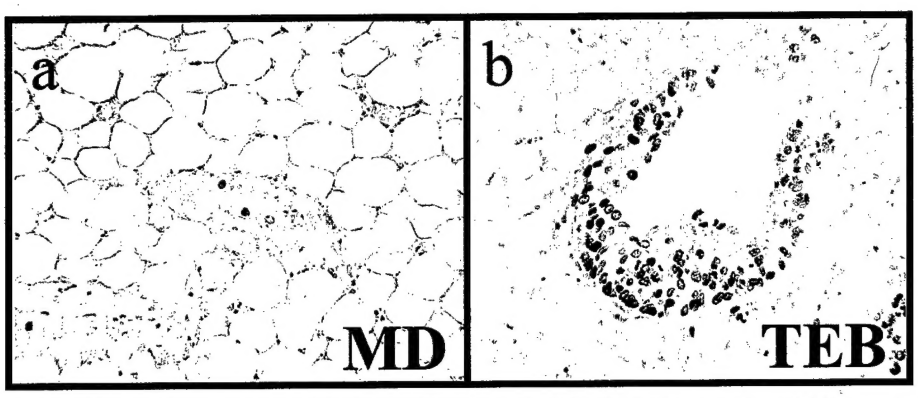


Fig.3 Immunostaining for BrdU in TEB's of mammary glands of adult(12 weeks old ) c-neu transgenic mice is unaffected by antiestrogen ICI 187, 780. Intact adult c-neu mice as is or after treatment with ICI 182, 780, daily for four days, were analyzed for immunoreactive BrdU as described previously (4).

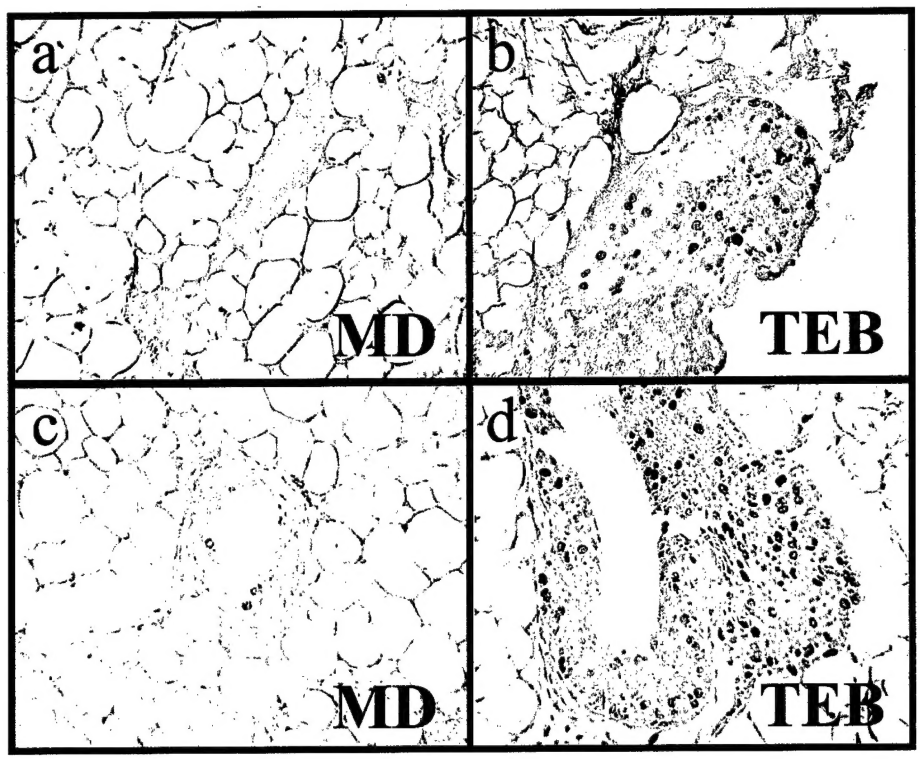


Fig. 4 Cell proliferation in teris of ovariactomized pubertal and adult c-neu transgenic mice is ovarian/estrogen independent. Mice were ovariactomized for 14 days prior to tissue removal and analysis for immunoreactive BrdU as described previously (4). Magnification, 400X. MD: mature duct; TEB: Terminal end bud.

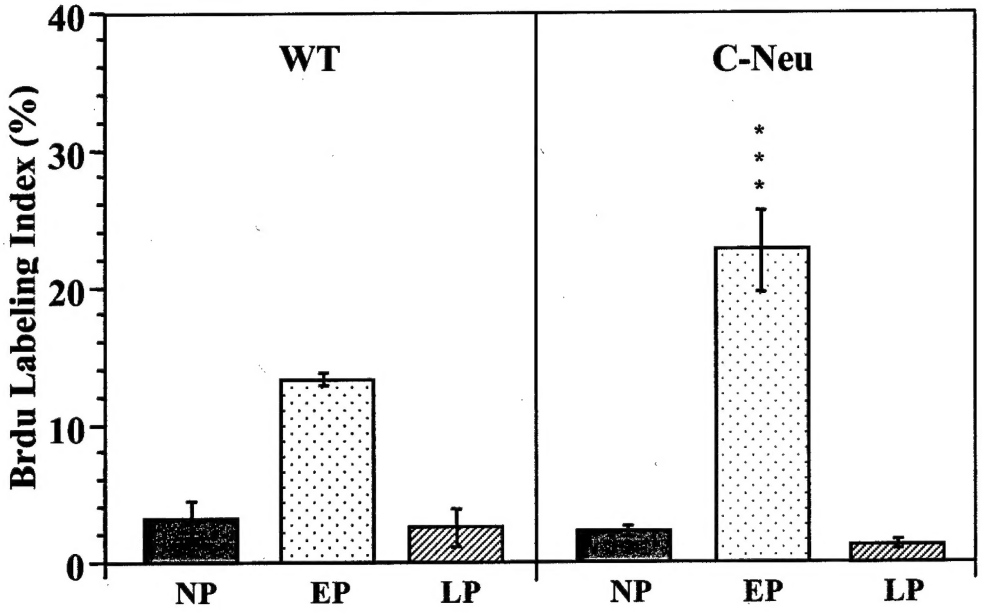


Fig. 5 BrdU postive cells in mammary glands of nulliparous and pregnant mice. Mammary glands from wild type (WT) and c-neu mice (C-Neu) were analyzed for BrdU positive cells as described previously (4). The data is presented as percentages (mean ±S.E.M.). For each experimental group, three mice were analyzed and mammary glands for each mouse were analyzed in triplicate. The percentage of immuno-positive cells was obtained by counting a minimum of 500 cells per gland. NP: Nulliparous; EP: day 6 of pregnancy; LP: day 18 of pregnancy.